TEMPORARY ALTERATION OF CHARACTER OF AN ORGANISM BELONGING TO THE COLON GROUP.

OSKAR KLOTZ,
Late Governor's Fellow in Pathology, McGill University, Montreal.

IN the spring of 1904 I was given the opportunity, through the kindness of my chief, Professor Adami, to study an organism isolated by him from the water of the St. Lawrence River. The results of these studies were published in the *Journal of Medical Research*,[1] and several interesting points in regard to the interagglutination of the Coli-Typhoid group were noted.

However, aside from the agglutination phenomenon, a peculiarity in the cultural characteristics was also observed. The microorganism on solid media resembled very much the appearance of colonies of B. coli. Grown on broth the microbe gave a stringy deposit, difficult to break up on shaking, and becoming more stringy on longer incubation. In litmus milk there was a primary acidity with a subsequent alkaline reaction of the medium, but no coagulation of the milk occurred. Indol was produced only after some weeks' incubation in Dunham's broth; and of the sugar broths, gas was produced most abundantly in the glucose medium. As was noted in my publication, the organism did not ferment lactose or saccharose when first isolated from the water, but did so after it had remained on the medium for some time. The saccharose broth was found to be more easily decomposed than the lactose, the latter medium showing only a very little gas formation after several days' incubation.

After the organism had been cultivated on artificial media for some months, the following experiment was reported:

A lethal dose of the bacillus was inoculated into the peritoneal cavity of a rabbit, and after its death (which resulted in three days), cultures were again obtained from it. The appearance of the organisms and the cultural characteristics were those of the bacilli inoculated, except that in the fermentation tubes there was a slight development of gas in the glucose broth, none in the lactose or saccharose. Transfers were made from these tubes into the respective sugar broths, that is, the glucose colony was transferred to glucose broth, the lactose colony to lactose broth, and the saccharose colony to the saccharose broth. After 24 hours' incubation there was an increased amount

[1] *Jour. Med. Res.*, 1904, 6, p. 475.

of fermentation in the glucose transfer, but still none in the lactose or saccharose. A second transfer was made, similar to the above, and now at the end of another 24 hours the glucose and saccharose broths were both fermented; no gas appeared, however, in the lactose. In both the glucose and saccharose there was also acid production. In the lactose broth no change was evident, though there was growth in the closed arm of the tube. Four days' incubation and transfer on lactose broth gave a small amount of gas formation, and transfers from this again into lactose led to its fermentation in 24 hours. The stock culture as control produced gas in all these sugar broths in 24 hours.

This feature of the organism, its variability in the power to break up certain sugars, presented two very interesting problems. First, are we justified in making an indefinite number of varieties of B. coli, depending on cultural characteristics which may be modified artificially; and secondly, in isolating from water an organism which in the first transfers does not ferment one or more definite sugars, but which, after remaining on artificial media for some time, acquires the property, can we conclude that the microorganism has recently had an animal host?

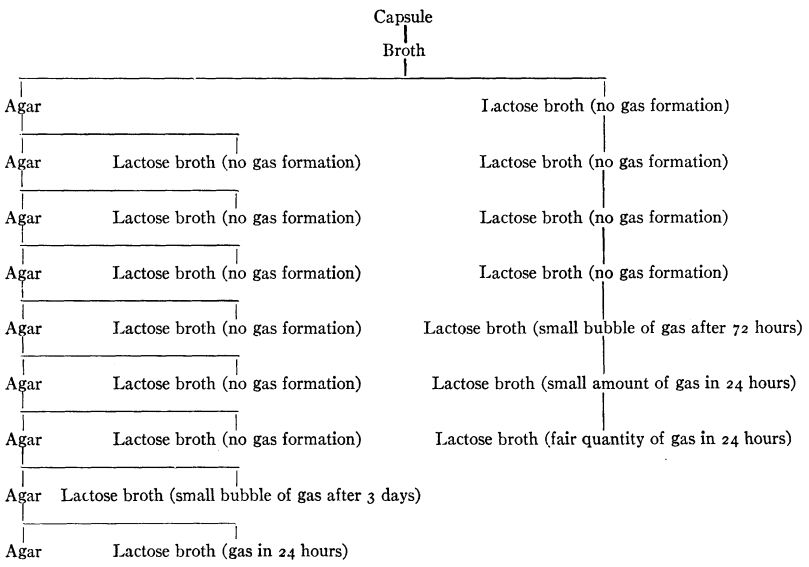
We have repeated our experiment of passing our microorganism, the Bacillus perturbans, through an animal. In this instance we made use of the celloidin capsules as devised by McCrae,[1] which we filled with a broth culture of the bacillus.

In the table it will be noted that two sets of transfers were carried forward, the one in which the parent culture was kept on sugar-free agar, and the other in which the parent stock was on a sugar medium similar to the transfer.

The sealed capsule was inserted, aseptically, into the abdominal cavity of a rabbit on September 19, 1904. The capsule was allowed to remain in the rabbit till February 10, 1905, in all 144 days. The capsule was then again obtained, and dropped for a moment without breaking into 10 per cent carbolic acid, after which it was placed in a flask of broth (without breaking), and incubated to insure against the chance of contamination. As no growth resulted, the capsule was ruptured and the microorganism was allowed to grow in weak broth for 18 hours. Transfers were then made into the various media, including sugar broths. The organisms resembled the original bacilli of the capsule in all the media except the sugars, though the growth was not in any case so luxuriant as transfers from the stock culture.

Transfers were made from and into the respective sugar broths daily. The glucose transfer showed a very slight gas formation on the first day, and this increased from day to day for several days. The first appearance of fermentation appeared in the saccharose on the fourth day, when it was only slight, and slowly increased with succeeding transfers. However, the lactose medium offered the greatest difficulty of fermentation, as can be readily appreciated from the following table.

[1] *Jour. Exp. Med.*, 1901, 5, p. 635.



As is seen in the above table, when the organism was transferred from a lactose to a lactose medium it regained its power to ferment lactose more rapidly than did the agar colonies. Having once acquired this property, the bacillus retained the lactose-splitting power in the successive transfers. In other words, the microorganism, having been deprived of one of its functions of altering the composition of certain materials by forced growth or environment, may again regain this function if it remain in contact with the material over an extended period.

Peckham[1] has given us the most complete study of the influence which environment exerts on the characters of organisms, especially of the colon group. In some cases this alteration consisted in an excessive activity of one function, in others the opposite, certain traits of the bacillus being entirely lost. In a series of cultural experiments, she was able to force B. typhosus to produce indol.

Of the external influences which can be brought to bear on bacteria, alteration of the quantity or quality of the food supply plays the most important rôle, and leads to modification of their biological nature. Thus some bacteria, in their normal metabolism, if we may so call the cell activity, secrete enzymes which split up proteids;

[1] *Jour. Exp. Med.*, 1897, 2, p. 540.

others secrete ferments acting on sugars. The colon bacillus, among others, possesses a proteolytic ferment, whose activity we estimate by the quantity of indol produced in the medium. If, however, the colon bacillus be grown over an extended time in river water, its power to produce indol is diminished or entirely lost; while again, as was said above, if a non-indol producing organism, such as the typhoid bacillus, be grown in a medium containing proteids alone, it acquires the property of producing indol.

Other examples of the influence of environment on bacteria are well known. Jenner[1] found that he could revert B. coli capsulatus to an unencapsulated form by cultural methods. The new variety then possessed characteristics dissimilar to the previous capsulated form; as for instance, while the capsulated bacillus coagulated milk, the unencapsulated stock lost this power when placed in this medium. A more remarkable difference was noted in the pathogenesis of these two varieties, for, as we know, B. coli capsulatus is very pathogenic for white mice, but becomes less fatal or even non-pathogenic on losing its capsule.

Experimenting with this same organism, B. coli capsulatus, Larulle[2] reports similar results of transforming his "opaque" variety into the "transparent," by passing the former through animals.

Other examples of alteration to a lesser degree in the characters of bacteria are seen in the everyday cultures, in the increase or decrease of the amount of acid produced, the morphological change which organisms undergo when inoculated on different media, and many other variations.

In a paper on the variability of bacteria Adami[3] discussed the alterations of character in bacteria due to environment, which give rise to different races of microorganisms. There he pointed out that two kinds of variations may occur, the temporary variation, in which the microorganism acquires characters that are lost after several transfers have been made, and the permanent variation, in which a new function or change is impressed on a microbe and remains with it in all future cultures. Of the latter class there are not many, for we must remember that what we call permanent is but a relative

[1] *Jour. Path. and Bact.*, 1898, p. 257.

[2] *La Cellule*, 1889, 5, p. 61.

[3] *Medical Chronicle*, 1892, 16, p. 366.

term. We speak of the characters as permanent when, after weeks, months, and years, no change is noted in the transfers from the type of the parent stock.

That at least temporary modifications can be brought about by such simple methods of cultivation and in so short a space of time seems to me to indicate that among those which we call varieties of B. coli there are some which owe their differentiating qualities to a prolonged habitat in a medium differing from that in which the parent stock has had its growth, and that through subsequent growth in suitable media the original qualities of the parent stock may be acquired. Our culture medium is at best a poor imitation of the natural habitat of these minute, and, I might say, impressionable, living bodies; hence we can conceive that investigators may obtain different results with the same organism. Thus with the colon bacillus it would seem that so long as we bring forward new sugars to ferment, we get an equal number of new varieties.

Further, when organisms, which under ordinary conditions produce gas in sugar media, are found to have lost this quality, it is one of the alternatives that the organism has been a parasite in the animal body. In our own case the same organism was also isolated from sewage flowing into the river, and the reactions of this strain of the microbe on media were the same as described, that is, it was primarily a non-lactose fermenter, but later acquired the property to break up this sugar.